www.ajbrui.org

Afr. J. Biomed. Res. Vol. 23 (January, 2020); 117- 122

Research Article

Effect of *Garcinia Kola (Heckel)* on Pharmacokinetic Parameters of Rifampicin

***Ayogu E.E.[1], Okpalaoka O.[1], Amorha K.C.[1], Nduka S.O.[2], and Okonta M.J[1]**

[1] *Pharmacokinetic Research Unit, Department of Clinical Pharmacy and Pharmacy Management, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001, Nsukka.*

[2] *Department of Clinical Pharmacy, Nnamdi Azikiwe University Awka, Anambra State*

ABSTRACT

Most herbal products are used with prescription drugs and have been found to affect the pharmacokinetics of these prescription drugs. The effect of concurrent administration of *Garcinia kola* on the pharmacokinetic parameters and penetration profile of rifampicin into the lung tissues were investigated using animal model. Albino rats in group A received rifampicin 10 mg/kg alone orally; Group B and C received 100 mg/kg and 200 mg/kg of *Garcinia kola* extract respectively for 10 days and on day 11, rifampicin 10 mg/kg was given. Blood samples were withdrawn from each group at 0.5, 1, 2, 4, 8, 12 and 24 h time intervals respectively. Blood samples were also withdrawn from the lungs in each group after 24 h and assayed to determine the concentration of rifampicin in the lungs**.** Both 100 and 200 mg/kg of *Garcinia kola* showed reduction in the maximum concentration (C_{max}), area under concentration (AUC), clearance time (Cl_T), and time for plasma concentration to decrease by half ($t_{1/2}$) of rifampicin. *Garcinia kola* significantly (P = 0.01) decreased concentration of rifampicin in the lungs by 32% and 39 % respectively. Our results show that the co-administration of *Garcinia kola* and rifampicin impairs the bioavailability of rifampicin and its penetration into the lungs.

Keywords: Garcinia Kola, Pharmacokinetics, Rifampicin

*Author for correspondence: *E-mail*: ebere.ayogu@unn.edu.ng; Tel: +234-8039307396

Received: March 2019; Accepted: October, 2019

Abstracted by:

Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Plants naturally produce large number of diverse bioactive compounds used for management of ailments (Altemimi *et al.*, 2017; Dranca and Oroian, 2016). Thus the use of herbs in disease management has historical and traditional background with a belief that they are safe (Mohammad and Mohammad, 2009). Traditional knowledge of the values of these plants were earlier downplayed by pressures of modern lifestyle, orthodox medicine and effects of rampant deforestation (Onyekwelu *et al*., 2015); However current researches has fanned into flame the indispensable use of plants in disease management. Recently, the rate of self-medication with herbs and food supplement is on the increase and this has led to greater potentials for interaction between these agents when administered concurrently with prescription drugs (Ulbricht *et al*., 2008). Though people believe that natural plants are safe, they contain active constituents in varying proportions which make them potential drugs as well as poisons. Of great concern is the fact that as herbal drugs and food supplement becomes more popular, the more the consumers, health professionals and manufacturers tend to overlook the issue of safety concerning them. It is on this ground that most of the commonly used herbs are under researched by researcher Clinical Pharmacokinetics is the use of principles derived from pharmacokinetics in the management of patient drug therapy. Basic parameters that influence these principles are Peak Plasma Concentration (C_{max}) and the time it takes to reach this peak (T_{max}). These two parameters are influenced by rate of absorption. Other factors include elimination half-life which is very useful in the calculation of drug dosage regimen; Volume of distribution which is simply a proportionality constant whose sole purpose is to relate the plasma concentration (Cp) and the mass of drug (X) in the body at a time. It is not a physiological volume". [4].Volume of distribution is very vital in determining how readily an administered drug is distributed between the body tissues and blood; Clearance which is an inevitable parameter in the calculation of loading dose and dose needed to maintain steady state concentration

In Africa, *Garcinia Kola* (Clusiaceae), known as bitter kola is a treasured multipurpose tree indigenous to West and Central Africa. Garcinia Kola tree is often referred to as "wonder plant" because all of its part is medicinal (Manourová *et al.*,2019; Onasanwo *et al*., 2016). Its seed has been used in several ways in the management of a good number of health problems (Ezeigbo *et al.*, 2016) which include liver disease, cold, cough aphrodisiac, voice coarseness (Farombi, 2011; Alabi et al., 2017), and neurodegenerative diseases such multiple sclerosis (Omotoso *et al.*, 2018).Other studies shows it has antibacterial activities against multi-drug-resistant *A. baumannii* (Badger-Emeka *et al*., 2018), anti-inflammatory and pain relieving effects (Ibironke *et al.*, 2015; Tchimene *et al.*, 2016)

Some of the phytochemicals compounds in *G. kola* include: oleoresin, tannin, saponin, alkaloids, cardiac glycoside,

flavonoids and metallic ions like aluminium, magnesium, calcium, copper. Its flavonoid extract - kolaviron possess anti-malarial, wound healing properties (Nwaehujor, *et al*., 2015; Tshibangu,*et al*., 2016), is effective in treatment of benign prostatic hyperplasia (Kalu, *et al.,* 2016) and can be clinically active against ischemia/reperfusion injuries (Akinmoladun *et al.*,2015)

Studies have shown that flavonoids and metallic ions form various intermolecular complexes with a variety of compounds (; Terashima *et al*., 2002, Esimone, 2002a). Earlier studies have shown that ethanol extract of *Garcinia kola* has activities against the antimicrobial properties of some commonly used antibiotics such as tetracycline, gentamycin, penicillin G, ofloxacin, co-trimoxazole and amoxicillin-clavulanic acid, when administered concurrently (Esimone *et al*., 2007; Esimone *et al*., 2002a; Esimone *et al*., 2003).

Rifampin is a semisynthetic antibiotic produced from *Streptomyces mediterranei*. It has a broad antibacterial spectrum, including activity against several forms of Mycobacterium. In susceptible organisms it inhibits DNA-dependent RNA polymerase activity by forming a stable complex with the enzyme. It thus suppresses the initiation of RNA synthesis. Rifampin is bactericidal, and acts on both intracellular and extracellular organisms. (William, 2001) Rifampicin is an antitubercular medicine which helps to treat tuberculosis and meningococcal meningitis. A common side effect of rifampicin includes reddish colouration of the urine, sweat, sputum and tears. WHO has recommended that tuberculosis (TB) should be treated with a combination of anti-TB drugs, preferably in fixed dose combinations (FDC) to enhance compliance. Rifampicin is one of the first-line and most important anti-TB drugs approved for use in combination with other anti-TB drugs for the treatment of TB. Earlier researchers have shown that rifampicin exhibits variable bioavailability upon administration, being less soluble and highly permeable. (Ashokraj *et al*., 2008). Hence worldwide, the recommended administration of rifampicin is before breakfast. Therefore, challenges such as suboptimal or failed treatment resulting from interaction with another drug, food or herb could occur (Neralla and Glassroth, 2003).

The most valued part of the tree is the seed, which is commonly eaten to prevent or cure gastric disorders and for their typical astringent taste (Manourová *et al.,* 2019). This is supported by the work of Oluwatosin *et al*., who reported that some people consume Garcinia kola seeds instead of cola nuts coming from Cola spp (Oluwatosin *et al.,* 2014). There are no reports of harmful effects resulting from overdosing with G. kola, (Konziase, 2015) hence it is safe for consumption. Esimone *et al*., 2003 has shown that in Nigeria, most patients on antibiotics therapy also consume *G. kola* habitually or as a result of the general belief that it has anti-infective properties. Therefore, this study was designed to investigate the effect of co-administration of *G. kola* extracts on the pharmacokinetic parameters and penetration profile of rifampicin into the lung tissue.

MATERIALS AND METHODS

Drug and Plant Material: Rifampicin (Sanofi-aventis Ltd, Lagos Nigeria) and *Garcinia kola* were used. Garcinia kola seeds used in this study were obtained locally in Nsukka (Southeast Nigeria), authenticated at the Department of Pharmacognosy, University of Nigeria, Nsukka and stored in their herbarium.

Animals: Albino rats (190-300g) of both sexes were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were monitored under light and dark cycle in metabolic well-ventilated rodent cubicle before commencement of the experiment for the purpose of acclimatization. Measures like separate housing by sex was taken to avoid conception. Food and water were allowed them within the period of monitoring but was withdrawn 12 h before the commencement of the experiment. The animals were handled according to internationally and locally approved protocols for experimental animals.

Experimental protocol

Extraction of Garcinia kola: The seeds of *G. kola* were washed, peeled, dried and pulverized in a milling machine into powder (gumming form). About 2 kg powder of the *G. kola* was placed into 10 L maceration bottle with 5 L of ethanol and was macerated for 72 h, after maceration, the solvent was sieved out with porcelain cloth and later with 22 cm filter paper. The extract was evaporated to dryness using rotary evaporator. The sample was collected and stored in refrigerator at 4 ◦C.

Beer-lambert's Plot: The standard solutions of Rifampicin was prepared in the concentration of 1 to 8 µg/ml and dissolved in 3 ml plasma. The plasma solutions were further diluted with 10 % v/v of methanol and determine the absorbance of the samples using UV spectrophotometer (Sp-6-450 UV/Vis Pye unicam). In the method, protein free samples were prepared by mixing each sample of 5ml plasma with equal volume of 10 %v/v of methanol; the samples were centrifuged and filtered (Onyishi *et al.,* 2014). The absorbances of the filtrates collected were determined at 375nm using spectrophotometer.

Administration of Garcinia kola/Rifampicin and determination of plasma drug concentrations in rats: Fifteen albino rats were divided into three groups: A, B and C. The experiment was carried out in 3 Phases. In Phase 1, Group A received 10 mg/kg of rifampicin alone orally. In Phases 2 and 3, Groups B and C received 100 mg/kg and 200 mg/kg of *G. kola* extract respectively for 10 days and on day 11, rifampicin 10 mg/kg was given as in group A. Blood samples were collected from each group at 0.5, 1, 2, 4, 8, 12 and 24 h time intervals respectively (Onyishi *et al*., 2014) for the determination of plasma drug concentration in the rats.

Determination of the effects of Garcinia kola on rifampicin penetration into the lungs: At the 24th h, following the oral administration of rifampicin and *G. kola* in each group, all the animals were sacrificed with chloroform and their lungs collected. Blood was also withdrawn from the lung area with their plasma collected after centrifugation. For the purpose of removing contaminated blood, non-bacteriostatic saline was used to rinse the lungs. Each lung was homogenized individually and centrifuged to collect fluid and stored in a refrigerator for preservation before assay.

Determination of pharmacokinetic parameters: Following a single oral administration of rifampicin, different pharmacokinetic parameters were determined using non-compartmental method as implemented in WinNonLin pharmacokinetic programs (version 5.0), (Pharsight Corporation, Mountain View California). Pharmacokinetic properties of rifampicin were determined for each animal.

Statistical Analysis

In analyzing this result, it was assumed that after administration, rifampicin followed first order kinetics. Results were expressed as mean ± standard deviation (SD) and statistical comparisons for differences among the treatment groups were made using the students' paired t-test and one way analysis of variance (ANOVA). Data were analyzed at 95 % confidence interval with statistical significance set at $P < 0.05$.

RESULTS

Effect of Garcinia kola (100 mg/kg) on rifampicin (10 mg/kg) plasma concentration in rats following a single oral administration of rifampicin: When rifampicin was administered alone, it attained its maximum concentration of 18.24 ± 1.38 µg/ml at 1 h, while co-administration with *G. kola* at 100 mg/kg achieved a maximum concentration of 16.58 ± 1.07 µg/ml at the same 1 h. The difference in the concentration of rifampicin alone and that of rifampicin and 100 mg/kg *G. kola* was statistically significant (P = 0.03). The concentrations 18.00 ± 0.80, 16.57 ± 0.45, 16.29 ± 0.42, 15.26 ± 0.33 and 11.30 ± 1.29 µg/ml were obtained at 2, 4, 8, 12 and 24 h respectively for rifampicin alone while concentrations of 15.92 ± 0.90, 15.05 ± 1.38, 13.92 ± 0.80, 9.57 ± 1.72 and 7.05.37 ± 1.29 µg/ml were obtained at 2, 4, 8, 12 and 24 h for rifampicin + 100 mg/kg *G. kola*. A graphical representation of rifampicin concentration (µg/ml) at different time intervals following oral administration of rifampicin alone (10 mg/kg) and in the presence of 100 mg/kg *Garcinia kola* is shown in Fig. 1.

Effect of Garcinia kola (200mg/kg) on rifampicin (10 mg/kg) plasma concentration in rats following a single oral administration of rifampicin: Administration of rifampicin alone attained the maximum concentration of 18.24 ± 1.38 µg/ml within 1 h, which was significantly difference (P = 0.02) compared to the maximum concentration of 16.28 ± 2.41 µg/ml obtained from rifampicin plus 200 mg/kg of *G kola* within the same 1 h. The concentrations of 18.00 ± 0.80, 16.57 ± 0.45, 16.29 ± 0.42, 15.26 ± 0.33 and 11.30 ± 1.29 µg/ml were obtained at 2, 4, 8, 12 and 24 h respectively for rifampicin alone while concentrations of 15.00 ± 1.58, 14.84 ± 1.51, 12.03±0.86, 9.80 ± 1.51 and 7.25 ± 1.81 µg/ml were obtained at 2, 4, 8, 12 and 24 h for rifampicin plus 200 mg/kg *G. kola*. A graphical representation of rifampicin concentration (µg/ml) at different time intervals following oral administration of rifampicin alone (10 mg/kg) and in the presence of 200 mg/kg *Garcinia kola* is shown in Figure 2. There was no statistical difference (P = 0.10) in the plasma concentration of rifampicin produced by 100 mg/kg of Garcinia kola compared to that of 200 mg/kg.

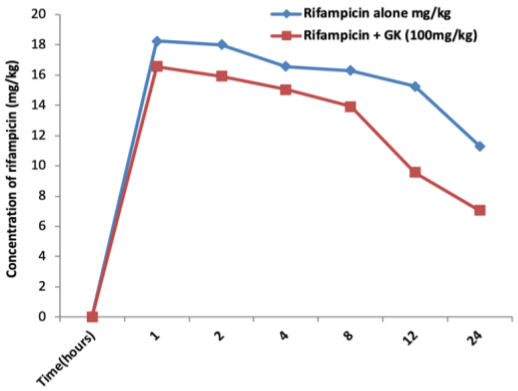

Figure 1:
A graph of mean plasma rifampicin concentration (µg/ml) at different time intervals following oral administration of rifampicin alone (10mg/kg) and in the presence of 100 mg/kg *Garcinia kola* seed extract.

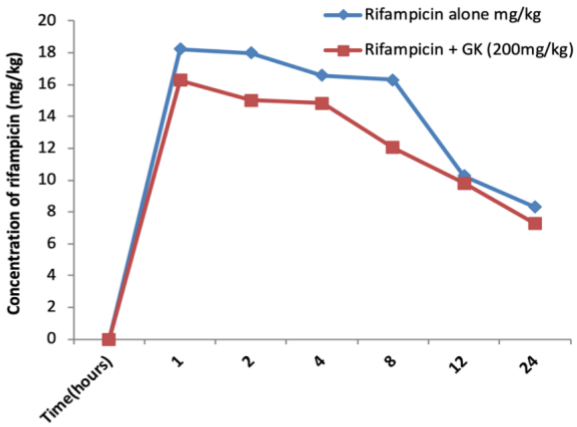

Figure:
A graph of mean plasma rifampicin concentration (µg/ml) at different time intervals following oral administration of rifampicin alone (10mg/kg) and in the presence of 200 mg/kg *Garcinia kola* seed extract

Table 1:
Pharmacokinetic parameters of rifampicin (10mg/kg) administered orally alone and Rifampicin+100 and 200 mg/kg of *Garcinia kola* in rats. (Mean ± SEM) (n = 5)

PARAMETER	RIF ALONE	RIF +100mg/kg GK (% change in parenthesis)	RIF + 200mg/kg GK (% change in parenthesis)
T_{max} (hr)	5.60 ± 4.60	5.4 ± 2.69 (3.57)	5.30 ± 1.09(5.36)
C_{max} (µg/ml)	18.60 ± 0.42	16.01 ± 0.52*(13.92)	15.08 ± 0.64(2.80)*
C_{last} (µg/ml)	17.30 ± 0.51	14.52 ± 0.47* (16.07)	16.76 ± 0.74(3.12)
AUC (µg/ml/hr)	377.35 ± 2.77	351.67 ± 4.38(6.80)	309.76 ± 28.4*(17.92)
$t_{½}$ (hr)	9.53 ± 2.80	1.26 ± 1.96*(86.78)	2.45 ± 1.21*(74.29)
V_d (ml/kg)	0.63 ± 0.04	0.60 ± 0.003(4.76)	0.54 ± 0.07(14.28)
C_L (ml/kg/hr)	0.009 ± 0.02	0.007 ± 0.04(22.22)	0.002 ± 0.04*(77.78)
AUMC (µg/ml/hr_2)	4739.05 ± 71.56	4257.50 ± 50.55(10.16)	2420.70± 88.64*(48.92)
MRT (hr)	12.57 ± 0.12	13.9 ± 0.138(-10.60)	15.8 ± 3.66(-25.69)

*SEM = standard error of mean; n = number of animal per group, * P < 0.05, The negative sign = decrease in parameter measured; RIF = Rifampicin; GK = Garcinia kola; T_{max} = time taken for drugs to attain maximal plasma concentration; C_{max} = maximal drug plasma concentration; C_{last} = measurable drug plasma concentration; AUC = area under concentration time curve from the time of dosing to the time of the last observation; AUMC = area under moment curve from the time of dosing to the time of last measurable concentration; MRT = mean residence time $t_{½}$ is terminal half-life; V_d = volume of distribution based on the terminal phase; C_L = total body clearance.*

Effects of 100 and 200 mg/kg Garcinia kola on Pharmacokinetics parameters of Rifampicin: The concurrent administration of both 100 and 200 mg/kg of *G. kola* showed a non-significant (P = 0.03) decrease in the T_{max} from 5.60 ± 4.60 to 5.4 ± 2.69 and 5.60 ± 4.60 to 5.30 ± 1.90 respectively. There was a significant (P = 0.02 decrease in the C_{max} and from 18.60 ± 0.42 to 16.01 ± 0.52 for 100 mg/kg G. kola and 15.08 ± 0.64 for 200 mg/kg G. kola. The $t_{1/2}$ was also significantly (P = 0.001 reduced from 9.35 ± 2.80 to 1.26 ± 1.96 for 100 mg/kg *G. kola* and to 2.45 ± 1.21 for 200 mg/kg *G. kola*. There was no statistically significant difference (P = 0.20) in the pharmacokinetic parameters of rifampicin produced by 100 mg/kg of Garcinia kola compared to that of 200 mg/kg. The effect of 100 and 200 mg/kg of *Garcinia kola* on other pharmacokinetic parameters of rifampicin are showed in Table 1

Effect of Garcinia kola on rifampicin penetration into the lung
Analysis of the result showed that concentration of rifampicin alone in the lung cavity was 14.63 ± 4.06 while that obtained from rifampicin+100 and 200 mg/kg *G. kola* were 9.81 ± 0.45 and 8.93 ± 0.5 respectively. The reduction in concentration was statistically significant at P = 0.01. A graphical representation of the effect of *Garcinia kola* on rifampicin penetration into the lung is shown in Figure 3.

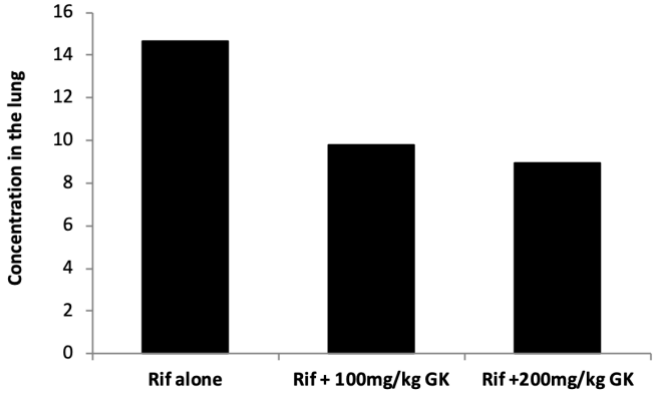

Figure 3:
Effect of 100 and 200 mg/kg *Garcinia kola* extracts on the rifampicin penetration into the lungs

DISCUSSION

The significance of food-drug interaction as a variable in bioavailability of drugs is now recognized. A major goal of an interaction study is to determine whether there is any increase or decrease in pharmacokinetic properties of a drug in the presence of an interacting substrate, because this has a potential to influence therapeutic outcomes. Hence the results of this work show that there is potential interaction between rifampicin and Garcinia kola when administered concurrently.

Both doses of *Garcinia kola* reduced the plasma concentration of rifampicin, which resulted in a decrease in maximum concentration (C_{max}) and AUC of rifampicin compared to the C_{max} and AUC observed with rifampicin alone. The mechanism by which *G. kola* elicits this effect was not investigated, but could be attributed to some factors relating to its constituents. Flavonoids and metallic ions are known to form intermolecular complexes with a wide range of compounds. Previous works on phytochemical analysis of *G. kola* shows that it contains flavonoids (Terashima *et al*., 2002, Esimone, 2002a) and metallic ions like aluminum, magnesium, calcium, copper (Esimone *et al*., 2003). The complex formed is usually non-absorbable which results in reduction in the amount of drug absorbed leading to reduced C_{max} and AUC. Also, food intake has been reported to increase the absorption of some drugs like sulphadimidine and griseofulvin while decreasing the absorption of others like penicillins and cephalexins (Iwu and Igboko, 1986).

In this study the presence of *G. kola* decreased the absorption hence the concentration of rifampicin which is indicative of reduced systemic bioavailability of rifampicin due to actual herb-drug interaction. This decrease in rifampicin C_{max} and AUC by *G. kola* is similar to the work by Igbinoba *et al*., who recorded decreased C_{max} and AUC of quinine by G. kola (Igbinoba et al., 2014). In the management of any disease, decrease in C_{max} of antibiotic leads to microorganism being exposed to sub-optimal concentrations with the possibility of drug resistance by the organism and consequent therapeutic failure.

There was a decrease in the time (T_{max}) for achieving C_{max} by rifampicin alone when compared to that of both the 100 and 200 mg/kg G. kola, while there was a significant decrease (P = 0.001) in $t_{1/2}$ in the treatment group compared to the group that took rifampicin alone. The decrease in T_{max} and $t_{1/2}$ suggests that *G. kola* affects both the rate and extent of rifampicin availability for absorption. This implies that the rifampicin dosage regimen needs to be adjusted downwards. Since $t_{1/2}$ is a major determinant in the calculation of drug dosage regimen, any drug that affects it will also affect the dosing of such drug.

The clearance of rifampicin was reduced in the treatment group of 100 mg/kg *G. kola* the reduction became significant (P < 0.01 with 200 mg/kg G. kola. The significant decrease in total clearance (C_L) suggests that *G. kola* may have induced decreased metabolism of rifampicin. It can be deduced that inhibition of liver metabolic enzymes played important role in the delayed elimination of rifampicin irrespective of the known potent liver enzyme induction by rifampicin. The treatment group also showed increase in the mean resident time (MRT) confirming delayed clearance. MRT is better appreciated when described as the residence time of individual molecules in the body. Each molecule of a drug spends a specific amount of time in the body, some lasting longer than the other, hence the need for calculation of the mean resident time molecules. Increase in MRT suggests that in the presence of *G. Kola*, some of its molecules still stay longer in the body than when given alone.

Reduced efficacy of tuberculosis therapy (TB) has been shown to be due to but not limited to factors such as high bacterial load and presence of resistant strain resulting to poor therapeutic outcome (Mitchison and Coates, 2004; Sacchetini *et al*., 2008). Analysis of the concentration of rifampicin in the lung tissue to assess the effect of *G. kola* on its penetration into the lungs revealed that *G. kola* caused a significant (P = 0.01) reduction on the concentration of rifampicin in the lung tissue. The plasma concentration of rifampicin for both rifampicin alone and rifampicin plus *G. kola* was higher than the concentrations obtained from lung tissues. Our result is in agreement with few studies published earlier indicating that in some remote target sites drug concentration can be reduced and the degree of reduction differs from drug to drug. (Din et al., 2017; Rizvi and Saleh, 2017).

The observed decrease in concentration of rifampicin in the lung tissue when taken concurrently with *G. kola* seed has various therapeutic implications and is of clinical importance especially in the management of *Mycobacterium* and *Staphylococcus* species with slightly sensitive strains. This is more likely to lead to therapeutic failure due to initial inadequate

concentration of antibiotic to totally eradicate the microorganism. It could also result to the presence of resistant strains of the organism due to exposure to suboptimal dose since previous studies have shown that the lung is the region where *Mycobacterium* organism inhabits (Kjellsson *et al*., 2012).

Ascertaining the concentration of administered drug in different cavities like the lungs is of paramount importance because some macrophages and neutrophils needed for the sustenance of *M. tuberculosis* are found growing in such region; hence maximum concentration is inevitable (Kaplan, 2003; Eum, 2010). Furthermore, poor prognosis and development resistance to tuberculosis treatment is associated with the presence and extent of organism growing in these cavities (Chang et al., 2004; Kim, 2008). Hence, any agent that affects the penetration of anti-TB agents into the lung is of great importance as it affects therapeutic goals.

Data analysis showed that there was no significant difference (P = 0.10) in the effect obtained from 100 mg/kg Garcinia kola compared to that of 200 mg/kg, hence the *G. kola* did not show a dose dependent effect.

In conclusion, our results reveal that *Garcinia kola* impairs the pharmacokinetic profile of rifampicin when administered concurrently. It reduced the C_{max}, AUC, $t_{1/2}$ and clearance of rifampicin resulting in decreased bioavailability. It also significantly reduced the concentration of rifampicin in lungs tissue. Hence, patients and health practitioners are advised against concurrent consumption of rifampicin and *G. kola.* If both of them should be taken, a time interval should be observed to avoid interactions.

REFERENCES

Akinmoladun, AC, Akinrinola, BL, Olaleye, MT, Farombi, EO (2015). Kolaviron, a Garcinia kola Biflavonoid Complex, Protects Against Ischemia/Reperfusion Injury: Pertinent Mechanistic Insights from Biochemical and Physical Evaluations in Rat Brain. Neurochem. Res., 40: 777–787. https://doi.org/10.1007/s11064-015-1527-z

Alabi, QK, Akomolafe, RO, Olukiran, OS, Adeyemi, WJ, Nafiu, AO, Adefisayo, MA, Omole, JG,Kajewole, DI, Odujoko, OO (2017). The Garcinia kola biflavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac. Pathophysiology, 24:281–290. https://doi.org/10.1016/j.pathophys.2017.07.003

Altemimi, A, Lakhssassi, N, Baharlouei, A, Watson, D, Lightfoot, D (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6:42-93 https://doi.org/10.3390/plants6040042

Badger-Emeka LI, Hany EK, Emeka PM (2018). Evaluation of Different Fractions of *Garcinia kola* Extracts against Multidrug Resistant Clinical Bacterial and Fungal Isolates. Pharmacogn J.;10(5):1055-60. doi : 10.5530/pj.2018.5.178

Chang KC, Leung CC, Yew WW, Ho SC and Tam CM (2004). A nested case-control study on treatment-related risk factors for early relapse of tuberculosis. *Am. J. Respir. Crit. Care. Med*., **170**:1124–1130.

Din F, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S,

Zeb A (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomedicine; 12: 7291–7309. doi: 10.2147/IJN.S146315

Dranca, F, Oroian, M (2016). Optimization of ultrasound-assisted extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC) from eggplant (Solanum melongena L.) peel. Ultrason. Sonochem., 3:637–646. doi: 10.1016/j.ultsonch.2015.11.008

Esimone CO, Adikwu MU, Ndu OO, Udeogaranya PO, Ezeugwu CO, Obonga W (2003). Effect of Garcinia kola seed extract on the antimicrobial properties of some antibiotics in-vitro. *J. Pharmaceutic. Allied Sci*. **2**: 114-120

Esimone CO, Nwafor SV, Okoli CO, Chah KF, Uzuegbu DB, Chibudu C, Eche MA, Adikwu MU (2002a). In vivo evaluation of interaction between aqueous seed extract of Garcinia kola Heckel and ciprofloxacin hydrochloride, *Am. J. Therap.*, **9**:275-280

Esimone CO, Nworu CS, Adikwu MU, Odimegwu DC, Ezugwu CO (2007). The effect of a new adaptogen, Garcinia kola seed, on the bioavailability of ofloxacin in humans. *Sci. Res. Essays*, **2**: 482-485,

Eum S *(2010).* Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. *Chest*, **137***:*122–128

Ezeigbo OR, Ejike EN, Nwachukwu I, Ejike BU (2016). Comparative Antibacterial Activity of Methanolic, Ethanolic and Aqueous Extract of Garcinia kola (Bitter kola) and Cola nitida (Kola nut). International Journal of Plant Research;6(3):53-56. doi: 10.5923/j.plant.20160603.01. **Farombi EO (2011).** Bitter Kola (Garcinia kola) Seeds and Hepatoprotection. In: Preedy VR, Watson RR, Patel VB eds., Nuts and Seeds in Health and Disease Prevention. Burlington, MA: Elsevier Inc., pp. 221–228.

Ibironke, G.F.; Fasanmade, A.A (2015). Analgesic and central nervous system depressant activities of kolaviron (A Garcinia kola biflavonoid complex). Afr. J. Biomed. Res., 18:217–223

Igbinoba SI, Onyeji CO, Akanmu MA, Soyinka JO, Pullela SSV, Cook JM, Nathaniel TI. Effect of Dehusked *Garcinia kola* Seeds on the Overall Pharmacokinetics of Quinine in Healthy Nigerian Volunteers. The Journal of Clinical Pharmacology 2014;20(20):1-7

Iwu MM and Igboko AO (1986). The flavonoids of *Garcinia kola. J Natural Prod*, **45**: 650-651.

Jambheker SS and Breen PJ (2012). Basic Pharmacokinetics 2nd ed. Pharmaceutical Press; London, pp. 32-56

Kalu,WO, Okafor, PN, Ijeh, II, Eleazu, C (2016). Effect of kolaviron, a biflavanoid complex from Garcinia kola on some biochemical parameters in experimentally induced benign prostatic hyperplasic rats. Biomed. Pharmacother., 83: 1436–1443. https://doi.org/10.1016/j.biopha.2016.08.064

Kaplan G (*2003).* Mycobacterium tuberculosis growth at the cavity surface: a microenvironment with failed immunity. *Infect. Immun.*, **71***:*7099–7108

Kim DH (2008). Treatment outcomes and long-term survival in patients with extensively drug-resistant tuberculosis. *Am. J. Respir. Crit. Care Med.*, 178:1075–1082

Kjellsson MC, Via LE, Goh A, Weiner D, Low KM, Kern S, Pillai G, Barry EF and Dartois V (2012). Pharmacokinetic Evaluation of the Penetration of Anti-tuberculosis Agents in Rabbit Pulmonary Lesions. *Antimicrob. Agent Chemother*., **56:**446-457

Konziase, B (. 2015). Protective activity of biflavanones fromGarcinia kola against Plasmodium infection. J. Ethnopharmacol, 172: 214–218 https://doi.org/10.1016/j.jep.2015.06.038

Manourová A , Leuner O , Tchoundjeu Z, Van Damme P, Verner V, Pribyl O, Lojka B (2019). Medicinal Potential, Utilization and Domestication Status of Bitter Kola (Garcinia kola Heckel) in West and Central Africa Forests, 10:124-142; doi:10.3390/f10020124

k**Mitchison DA and Coates AR (2004).** Predictive in vitro models of the sterilizing activity of anti-tuberculosis drugs. *Curr Pharm. Des.*, **10**:3285–3295

Mohammad Y and Mohammad I (2009) Herb-drug interactions and patient counseling. *Int. J. Pharm. & Pharm. Sci*, **1**:1

Neralla S, Glassroth J (2003). Mycobacterium tuberculosis: the treatment of active disease. *Semin. Respir. Infect.*, **18**:392-306

Nwaehujor, CO, Udegbunam, RI, Ode, JO, Udegbunam, SO (2015). Analgesic anti-inflammatory anti-pyretic activities of Garcinia hydroxybiflavanonol (GB1) from Garcinia kola. J. Korean Soc. Appl. Biol. Chem., 58:91–96. https://doi.org/10.1007/s13765-015-0011-4

Oluwatosin, A.; Tolulope, A.; Ayokulehin, K.; Patricia, O.; Aderemi, K.; Catherine, F.; Olusegun, A (2014). Antimalarial potential of kolaviron, a biflavonoid from Garcinia kola seeds, against Plasmodium berghei infection in Swiss albino mice. Asian Pac. J. Trop. Med., 7: 97–104. https://doi.org/10.1016/S1995-7645(14)60003-1G

Omotoso, GO, Ukwubile, II, Arietarhire, L, Sulaimon, F, Gbadamosi, IT (2018) Kolaviron protects the brain in cuprizone-induced model of experimental multiple sclerosis via enhancement of intrinsic antioxidant mechanisms: Possible therapeutic applications? Pathophysiology, 25: 299–306. https://doi.org/10.1016/j.pathophys.2018.04.004

Onasanwo, S.A.; Rotu, R.A (2016). Antinociceptive and anti-inflammatory potentials of kolaviron: Mechanisms of action. J. Basic Clin. Physiol. Pharmacol., 27:363–370. doi: 10.1515/jbcpp-2015-0075

Onyekwelu, J C, Olusola, J A, Stimm, B, Mosandl, R, Agbelade, A.D. (2015). Farm-level tree growth characteristics, fruit phenotypic variation and market potential assessment of three socio-economically important forest fruit tree species. Forests, Trees and Livelihoods, 24(1), 27-42. https://doi.org/10.1080/14728028.2014.942386

Onyishi IV, Chime SA, Ogudiegwu EO (2014). Formulation of novel sustained release rifampicin-loaded solid lipid microparticles based on structured lipid matrices from Moringa oleifera. *Pharm. Dev. Technol.*, **25**:1-9

Rizvi SAA, Saleh AM (2017). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal*; 26 (2018): 64–70

Sacchettini JC, Rubin EJ and Freundlich JS (2008). Drugs versus bugs: in pursuit of the persistent predator Mycobacterium tuberculosis**.** ***Nat. Rev. Microbiol*****., 6:41–52**

Tchimene KM, Anaga AO, Ugwoke CEC, Ezugwu CO, Okunji C, Iwu MM (2016). Bio-flavonoids and Garcinoic Acid from Garcinia kola seeds with Promising Anti- Inflammatory Potentials. Pharmacognosy Journal;8(1):56-58. doi:10.5530/pj.2016.1.12

Terashima, K., Y. Takaya and M. Niwa, (2002). Powerful antioxidative agents based on garcinoic acid from *Garcinia kola*. *Bioorganic. Med. Chem*., **10**:1619-1625.

Tshibangu, PT, Kapepula, PM, Kapinga, MJK, Lupona, HK, Ngombe, NK, Kalenda, DT, Jansen, O, Wauters, JN, Tits, M, Angenot, L, et al (2016). Fingerprinting and validation of a LC-DAD method for the analysis of biflavanones in Garcinia kola - based antimalarial improved traditional medicines. J. Pharm. Biomed. Anal.128: 382–390. doi: 10.1016/j.jpba.2016.04.042

Ulbricht C, Chao W, Costa D, Rusie-Seamon E, Weissner W and Woods J (2008). Clinical evidence of herb–drug interactions: a systematic review by the natural standard research collaboration. Curr. Drug Metab; **9**:1063–1120. **William Petri:** Tuberculosis In Goodman and Gilman's (ed) The pharmacological Basis of therapeutics. McGraw Hill Medical Publishing Division, 2001 10th Ed. p1277

Yasvanth Ashokraj, Kanwal Jit Kaur, Inderjit Singh, Gunjan Kohli, Shantaram Ramdoss Bhade, Manthena Varma, Shrutidevi Agrawal & Ramesh Panchagnula (2008) In Vivo Dissolution: Predominant Factor Affecting the Bioavailability of Rifampicin in its Solid Oral Dosage Forms, Clinical Research and Regulatory Affairs, 25(1):1-12.